

# B-cell depletion in rheumatoid arthritis: the prospect of long-term benefit

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B-cell depletion based on rituximab has now been proven to be an effective therapy for seropositive rheumatoid arthritis with a good safety profile. The encouraging results from early, open-label trials have been confirmed in three large, controlled trials. A license for use in patients that have failed antitumor necrosis factor- $\alpha$  agents has been obtained in the USA and is imminent in several other countries. Despite this welcome progress, experience suggests that all patients who respond to B-cell depletion eventually show clinical relapse. Possible mechanisms of relapse include incomplete depletion of pathogenic B cell clones, persistence of long-lived plasma cells producing pathogenic autoantibodies, or persistence of other kinds of cells, such as autoreactive T cells carrying 'disease memory'. A better understanding of why patients relapse may allow us to optimize B-cell depletion protocols based on rituximab and/or other agents and contribute to the development of B-cell-targeted therapies, with the ultimate goal of long-term remission.

Rheumatoid arthritis (RA) is a chronic inflammatory disease that involves predominantly diarthrodial joints and tendon sheaths. Its cause is unknown and the mechanisms of initiation and perpetuation of inflammation are not yet fully identified. Rituximab is a monoclonal antibody directed against the CD20 antigen that is very effective in depleting malignant and normal B cells *in vivo*. The efficacy and safety of B-cell depletion therapy based on rituximab for seropositive RA was established in 2002 by a pivotal Phase IIa randomized controlled trial [1]. Rituximab has recently been approved by the US FDA for the treatment of patients with RA refractory to antitumor necrosis factor (TNF) therapy.

When B-cell depletion was first used for rheumatoid arthritis, at University College London (UCL), the intention was to induce a long-term remission. Experience with 65 cases over 7 years indicates that, eventually, all patients will relapse and long-term remission is unachievable, even though patients have remained well for periods up to 44 months following one course of treatment [2]. Patients did not relapse during the period of B-cell depletion. Clinical relapse occurred either at the time of B-cell return to the peripheral blood or at a variable time, up to 32 months, after B-cell return [2]. These results suggest that although it may be possible to keep patients in remission by repeated B-cell depletion with rituximab, this may carry a risk of progressive immunosuppression, particularly in those cases that need to be maintained B lymphopenic continuously. In the last 4 years, research at UCL has focussed on possible

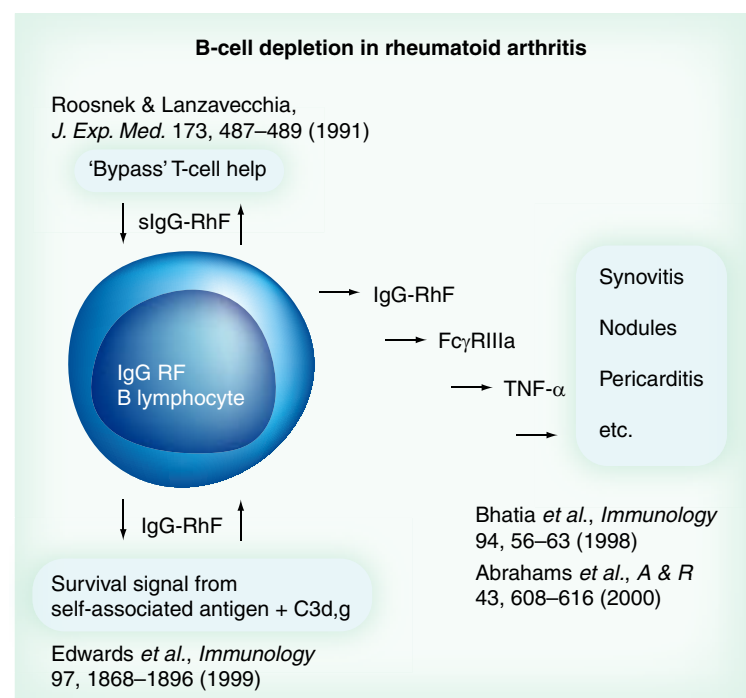
mechanisms of relapse. A better understanding of what causes patients to relapse may allow optimization of B-lymphocyte depletion protocols based on rituximab, perhaps in combination with newer agents, and eventually contribute to the development of B-cell targeted therapies capable of inducing truly long-term benefit.

## B cells in the pathogenesis of rheumatoid arthritis

The role of B cells in the pathogenesis of RA is controversial. Research at UCL into the role of synovial microenvironments in the pathogenesis of RA led in the late 1990s to an hypothesis in which B cells had a central role in both the initiation and perpetuation of the inflammation in RA [3–7]. Briefly, it was suggested that tissue pathology could be explained by the presence of certain pathogenic species of disease-associated autoantibodies, in particular, rheumatoid factors (RhF) of immunoglobulin (Ig)G isotype (IgG-RhF), able to form small immune complexes capable of evading complement clearance and activating macrophages through interaction with Fc $\gamma$ RIIIa (Figure 1). It was suggested that there was no need for autoreactive T cells to initiate the process as RhF-committed B cells are able to obtain T-cell help without need for loss of T-cell tolerance to IgG [8]. In addition, the capacity of IgG-RhF to form self-complexes with consequent binding of C3d provides a mechanism for delivering a second survival signal to RhF-committed B cells (by interacting simultaneously with the B-cell receptor and CD21). This would allow for such B-cell clones, once generated by

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**Figure 1. Early working hypothesis on the role of B cells in the pathogenesis of rheumatoid arthritis.**

B cells both as producers of autoantibodies capable of forming small immune complexes that can activate macrophages through interaction with Fc $\gamma$ RIIIa and as producers of autoantibodies that can lead to aberrant interactions between B and T cells.

Ig: Immunoglobulin; RhF: Rheumatoid factor; TNF: Tumor necrosis factor.

chance Ig gene rearrangement and mutation, to maintain their own survival indefinitely, effectively becoming self-perpetuating.

This hypothesis led to the idea that if it was possible to deplete the pathogenic B-cell clones and eliminate the pathogenic autoantibody species, it might be possible to induce long-term remission in patients with RA.

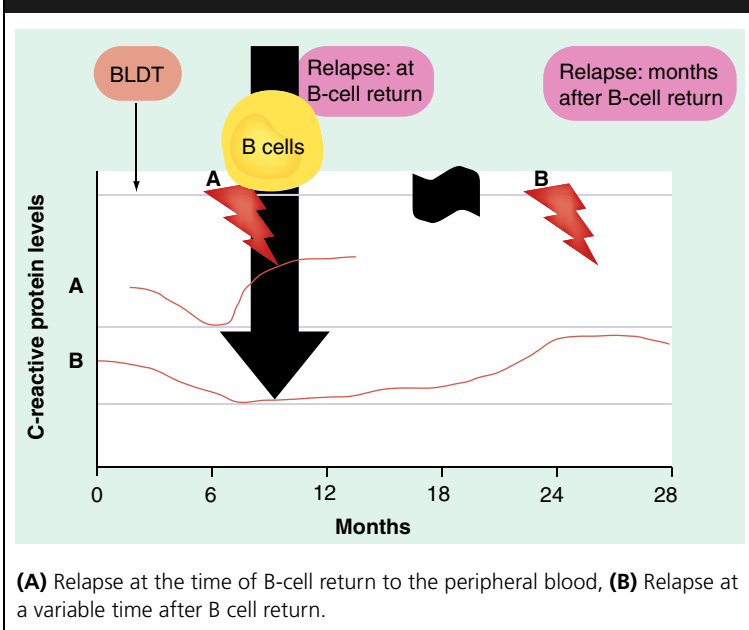
The subsequent success of B-cell depletion therapy in the treatment of rheumatoid arthritis, providing evidence to suggest that disease perpetuation is B-cell-dependent in a significant number of cases, has led to a major increase in interest in the possible roles of B cells in the pathogenesis of this disease. Possible roles suggested include activation of putative autoreactive T cells with inflammatory effector roles through means such as antigen presentation and cytokine production. While such roles cannot be excluded, it is not clear that they provide a viable rationale for B-cell depletion therapy, since the prediction might be that long-term B cytopenia would be needed to maintain remission. The original justification for B-cell depletion was on the basis that

a short period of depletion might, by removing pathogenic clones, 'restore tolerance' long term. It is conceivable that some sort of resetting of tolerance of putative autoreactive T cells might also be achieved by short-term B-cell depletion, but the theoretical basis for this is unclear.

### B-cell depletion in the treatment of rheumatoid arthritis

Specific B-cell depletion was made possible by the availability of rituximab, an anti-CD20 monoclonal antibody licensed for the treatment of B-cell non-Hodgkin's lymphoma that had proved to be very effective in the depletion of normal and malignant B cells *in vivo*. The first five patients with active, refractory RA were treated with a combination protocol that included rituximab, cyclophosphamide and oral prednisolone [9]. This protocol was used because trials in patients with lymphoma had shown that addition of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) to rituximab monotherapy increased the response rate from approximately 50% to more than 90% [10]. All five patients with RA achieved major improvement in symptoms and signs of active disease [9]. In two of these patients, clinical relapse was coincident with B-cell return to the peripheral blood, while in the other three it occurred only 10–17 months after B-cell return. This early open-label trial was then extended to a total of 22 patients, with results suggesting a dose response to rituximab [11]. Again, patients relapsed only after B-cell return to the peripheral blood, either at or very close to B-cell return or several months afterwards (Figure 2).

The encouraging results from both these open-label trials led to the Phase IIa double-blind controlled trial that provided definitive evidence for the efficacy of rituximab-based protocols in the treatment of active seropositive RA [1]. In patients treated with rituximab alone under corticosteroid cover in the context of continued methotrexate therapy, or in patients treated with rituximab and cyclophosphamide under corticosteroid cover with withdrawal of methotrexate at baseline, the benefit observed was comparable to that reported for anti-TNF- $\alpha$  agents [1]. Between 65% and 76% of patients treated with rituximab achieved at least an American College of Rheumatology (ACR)20 grade of improvement sustained at 6 months. Two further large controlled trials have confirmed the efficacy of rituximab-based therapy [12,13].

**Figure 2. Patterns of relapse following B-cell depletion.**

#### Possible mechanisms of relapse in rheumatoid arthritis following B-cell depletion

Experience has shown that eventually all patients relapse following an earlier response to B-cell depletion therapy, even though patients can sustain responses up to many months after B-cell return to the peripheral blood [2]. Several mechanisms may contribute to relapse, including incomplete depletion of pathogenic B-cell clones, persistence of long-lived plasma cells producing pathogenic species of autoantibodies capable of providing re-afferent signals to new B cell clones, or to the presence of other cells, such as autoreactive T cells, in which disease memory may reside.

#### *Incomplete depletion of pathogenic B-cell clones*

Rituximab induces an almost complete depletion of B cells in the peripheral blood (frequently by more than 99% of baseline values) for a period that usually lasts between 6 and 9 months [14]. Little is known about the exact degree of depletion of normal B cells in solid tissues, including bone marrow and secondary lymphoid tissues (lymph nodes and spleen) in humans following treatment with rituximab [15]. Animal studies in primates suggest that depletion in solid tissues is significant but incomplete and that it varies from site to site and in different individuals, even if treated with the same dose of anti-CD20 antibody [16,17]. Studies in mice

rendered transgenic for human CD20 suggest that different B-cell subpopulations have different sensitivities to killing by anti-CD20 monoclonal antibodies, probably related to innate and acquired survival characteristics of the cells as well as to the characteristics of their microenvironment [18]. Additional factors include their relation to anti-CD20 pharmacokinetics and pharmacodynamics (concentration achieved in different tissues, availability and effectiveness of effector mechanisms recruited by anti-CD20) [19]. Follicular B cells were most susceptible to killing by anti-CD20, compared with marginal zone B cells, which were relatively resistant, and B cells undergoing germinal center reactions at the time of anti-CD20 administration, which were the most resistant to killing [18].

At UCL, the extended open-label trial in 22 patients suggested a dose-response to rituximab, as did the most recent dose-ranging controlled trial [11,12]. This suggests that current doses may not be at the top of the dose-response curve, although it is unclear whether major additional benefit should be expected at higher dosages [12]. Additionally, patients at UCL who did not deplete well in the peripheral blood did not respond to treatment [11,14]. Immunophenotypic studies showed that patients who relapsed at the time of B-cell return to the peripheral blood tended to have a higher frequency of B cells with a memory phenotype when compared with patients who relapsed only later [14]. This suggested that the degree of depletion of memory B cells in solid lymphoid tissues might have been less in the group of patients who relapse earlier. Furthermore, in a small cohort of RA patients treated with rituximab, there was a trend to longer duration of clinical response in patients with evidence of more complete depletion in bone marrow aspirate samples 3 months after treatment [20].

Interestingly, follow-up of patients receiving repeated cycles of therapy suggests that there are parameters of disease dynamics which vary between patients but which remain relatively constant for a given patient. Individual patients showed similar dynamics of relapse, either at the time of B-cell return to the peripheral blood or at a variable time afterwards, following each repeated course of treatment [11,14,19]. Differences between patients may be due to differences in the load of pathogenic B-cell clones, differential pathogenic potential of autoantibodies associated with B-cell clones, differential presence of B-cell clones that are more resistant to killing by rituximab (due to their maturation status or the characteristics of

their microenvironment) or differences in host effector mechanisms recruited by rituximab to kill B cells (antibody-dependent cellular cytotoxicity and complement-induced cytotoxicity).

It was also observed that patients can repopulate fast with peripheral blood CD19-positive (B) cell counts reaching the normal range within weeks or that they can repopulate slowly only reaching a normal CD19-positive cell count after several months [14,19]. There was no correlation between the pattern of relapse following B-cell depletion and the speed of B-cell repopulation in the peripheral blood, patients with slow and rapid patterns of repopulation relapsing both at the time of B-cell return or later. The time to B-cell return to the peripheral blood and the speed of repopulation of the peripheral blood are most likely determined by the degree of depletion achieved, the clearance of rituximab allowing normal proliferation and full maturation of B-cell precursors in the bone marrow and by the individual regenerative capacity of the bone marrow B-cell precursors and microenvironment [20].

***Presence of long-lived plasma cells producing pathogenic species of autoantibodies***

Normal plasma cells do not express CD20 and are therefore not depleted by rituximab (Figure 3). Following B-cell depletion with rituximab, plasma cells with a short half-life will die and, in general, will not be replaced, as their precursors

will have been depleted by the anti-CD20 antibody. It is also possible that even if the precursor B cells have not been completely depleted, the presence of rituximab for a long period following treatment will limit the cells' capacity of proliferation and further differentiation into plasma cells.

By contrast, plasma cells with a relatively longer life will continue to be present and to produce antibodies. If plasma cells producing RhF survive for longer than 6–9 months, the usual period of B-cell depletion following treatment with rituximab, the availability of the autoantibodies they produce could potentially contribute to the aberrant survival of newly formed naïve RhF-specific B cells by providing their own antigen in the form of small immune complexes associated with C3d, leading to clinical relapse. Thus, residual plasma cells may be able to 'educate' new B cells into a self-perpetuating role and reignite disease.

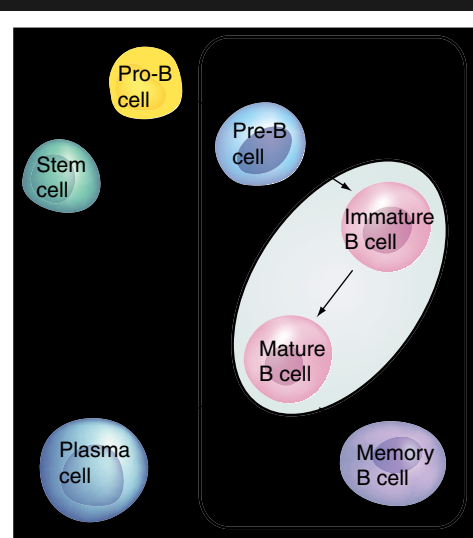
Serological studies at UCL showed that IgA-, IgG- and IgM-RF and IgG anticyclic citrullinated peptides (CCP) antibodies decreased significantly following B-cell depletion and that, for IgA and IgG autoantibodies, the decrease was proportionally more than that observed in the respective total serum Ig levels, and in the antimicrobial antibodies (IgG antitoxoid and IgG antipneumococcal capsular polysaccharides antibodies) [21]. These results suggested a selective effect on autoantibodies, possibly due to the fact that a significant proportion of the disease-associated autoantibodies are produced by relatively short-lived plasma cells. Disease-associated autoantibodies decreased more in the group of patients who responded to treatment than in the group of patients who did not [21].

These serologic studies also showed that only rarely did IgA-, IgG- and IgM-RF and anti-CCP antibodies decrease to within the normal range or become undetectable, suggesting that a proportion of autoantibody-producing plasma cells have a longer half-life. Animal studies have showed that anti-double stranded (ds)DNA antibody-producing plasma cells can be long-lived [22].

***The memory for the disease residing in other cells, such as autoreactive T cells***

Following B-cell depletion with rituximab, clinical relapse is more associated with a rise in autoantibody serum levels than with the presence of B cells *per se* [21]. In the UCL, and in other small cohorts, only patients seropositive for RhF have responded to B-cell depletion [2,11,23]. This suggests that the central role that B cells play in

**Figure 3. Expression of the CD20 antigen on cells of B-cell lineage.**



Cells expressing CD20 are susceptible to killing by rituximab.

the perpetuation of inflammation in at least a subgroup of patients with RA is mediated through their production of autoantibodies. Autoantibodies may have direct roles in inducing inflammation but may also facilitate aberrant interactions between B and T cells. These observations do not exclude the possibility that clinical relapse following B-cell depletion therapy may be due to the presence of autoreactive T cells that are able to re-initiate the process when B cells with the appropriate specificity are available [24].

In the latest rituximab trials it has been reported that some patients with RA seronegative for RhF have responded to treatment [13]. This raises the possibility that B cells may also contribute to inflammatory disease by supporting autoreactive T cells with inflammatory effector function, through presentation of antigen and cytokine production. The puzzle here is that if B cells' main pathogenic role was antigen presentation to T cells, you would still expect these B cells to secrete autoantibodies [24]. However, it can also not be excluded that, in RhF seronegative patients, autoantibodies of different specificities, for which detection systems are currently unavailable, may be involved in the disease pathogenesis.

Finally, disease relapse following B-cell depletion not due to insufficient depletion of pathogenic B-cell clones or of pathogenic autoantibody-producing plasma cells could also be due to a primary defect in mechanisms that regulate central or peripheral B-cell tolerance, leading to a new but still pathogenic B-cell repertoire at reconstitution.

### **Mechanisms of relapse & development of further B-cell targeting therapies**

A better understanding of the mechanisms of relapse in RA following response to B-cell depletion would allow us to design potentially more effective B-cell targeting therapies. If the main mechanism for relapse is incomplete B-cell depletion, then higher doses of rituximab, more effective B-cell depleting agents or combination therapy may lead to better results.

Combination therapy may be especially important, to target subpopulations of B cells whose innate or acquired survival mechanisms or microenvironment allow them to resist killing by rituximab. Ideally, one would hope that pathogenic B-cell clones would be more dependent on certain mechanisms for their development, expansion and survival, allowing the development of more selective therapies with potentially higher efficacy and less side

effects. Several agents have been developed, which aim to neutralize B-lymphocyte stimulator protein (BLyS; BAFF), a soluble survival factor important for both early B-cell development and the transition of B cell to plasma cell [25]. Some of these agents also neutralize a sister cytokine, APRIL, which also has effects on B-cell survival, although these are less well understood. An antibody to BLyS, Lymphostat B, has been tested in Phase I and II trials in RA and has shown significant effects on B-cell numbers and antibody profiles [26]. However, clinical efficacy appears to be modest for monotherapy. Three receptors are known to bind either BLyS (BAFF-R or BR3) or both BLyS, and APRIL (TACI and B-cell maturation antigen [BCMA]), and Ig Fc fusion proteins based on these receptors are under investigation (BR3-Ig, TACI-Ig and BCMA-Ig) [27]. There is some early evidence for biological effects as for Lymphostat B, but again, it is unclear that sequestration of BLyS with these agents alone will have major clinical efficacy. Nevertheless, studies in mice by Chan and colleagues suggest that in combination with anti-CD20, BLyS blockade may allow a much more complete depletion of solid tissue B cells [18]. CD22 is a protein found on the surface of B cells with negative signaling properties. Anti-CD22 monoclonal antibodies with agonist effects may prove to be useful B-cell targeting therapies alone or in combination with anti-CD20 antibodies [28]. A further possible strategy under investigation is the blockade of interleukin (IL)-21, which is involved in the generation of new plasma cells from naïve and memory B cells, particularly for T-cell dependent antigens [29].

If relapse is due to the presence of longer-lived plasma cells producing pathogenic species of autoantibodies, combination therapy with agents targeting both B cells and plasma cells might be more effective. This approach may be limited by the availability of an effective agent to selectively kill fully-differentiated plasma cells and the potential higher risk of infections associated. Inhibition of IL-21 may block the generation of plasma cells but may not affect existing plasma cells [29]. Targeting BCMA, one of the receptors for BLyS and APRIL, may be useful, as BCMA is selectively expressed by plasma cells [30].

If the memory for the disease resides in other types of cells, such as autoreactive T cells, then combination therapies with agents targeting B cells and agents targeting other cells or their

interaction with B cells, such as cytotoxic T-lymphocyte antigen (CTLA)4-Ig (abatacept), may be able to induce long-term remission [31].

Conclusion

B-lymphocyte depletion based on rituximab for the treatment of RA was met with scepticism when first proposed, but has since been proved to be an effective therapy with a good safety profile. It was designed based on a hypothesis attributing a central role for B cells in the pathogenesis of RA through the production of specific pathogenic species of autoantibodies. Evidence gathered since that time remains consistent with this general concept. Clinical response is more closely associated with changes in autoantibody serum levels than with the presence or absence of B cells *per se*, suggesting that the primary pathogenic role of B cells is mediated through the production of autoantibodies. However, the precise detail of what may prove to be a multilayered etiopathogenic process remains unknown. The original

hope of long-term remission has been only partially fulfilled, but ongoing studies of mechanisms of relapse may provide a rationale for more effective and long-lasting B-cell targeted therapies.

Future perspective

The efficacy of B-cell depletion therapy in RA and in other autoimmune diseases has shown that, in many of these patients, B cells can have a central role in disease perpetuation. This has led to an exponential increase in research on possible roles of B cells in the pathogenesis of the different autoimmune diseases in which B-cell depletion therapy has produced positive clinical results. We believe that this will eventually lead to a better understanding of the pathogenesis of RA as well as of other autoimmune diseases, of the mechanisms underlying the effectiveness of B-cell depletion in the treatment of these diseases, and, eventually, result in the development of more effective and, possibly safer therapeutic strategies.

Executive summary
<b>B-cell depletion in rheumatoid arthritis</b> <ul style="list-style-type: none"><li>• B-cell depletion based on rituximab has been proved to be an effective therapy for seropositive rheumatoid arthritis (RA) with a good safety profile in a Phase II controlled trial. Further Phase III trials have confirmed these results.</li></ul>
<b>Clinical relapse following B-cell depletion</b> <ul style="list-style-type: none"><li>• The earlier objective of long-term remission has not been achieved with current protocols.</li><li>• At University College London (UCL), patients did not relapse during the period of B-cell depletion.</li><li>• Patients relapsed either at the time of B-cell return to the peripheral blood or at a variable time after B-cell return.</li><li>• Relapse was more closely associated with a rise in disease-associated autoantibody serum levels than with the presence of B cells <i>per se</i>.</li></ul>
<b>Possible mechanisms of relapse in rheumatoid arthritis following B-cell depletion</b> <ul style="list-style-type: none"><li>• Incomplete depletion of pathogenic B-cell clones.</li><li>• Presence of long-lived plasma cells producing pathogenic species of autoantibodies.</li><li>• The memory for the disease residing in other kinds of cells, such as autoreactive T cells.</li></ul>
<b>Mechanisms of relapse and development of further B-cell depleting and B-cell modulating therapies</b> <ul style="list-style-type: none"><li>• A better understanding of the mechanisms underlying clinical relapse in RA may lead to optimization of rituximab-based protocols and to development of new and possibly more effective B-cell targeting or combination therapies.</li></ul>

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